

Effect of Low-temperature Plasma on Polysaccharide Components of *Pleurotus ostreatus* Fungi

Xiaoyan Xu¹, Yan Guo¹, Youjun Wang¹, Jie Zhu^{1,2,*}

¹ School of Pharmacy, Changzhou University, Changzhou Jiangsu, 213164, China

² Institute of Urban & Rural Mining, Changzhou University, Changzhou Jiangsu, 213164, China

* Corresponding author: Jie Zhu (Email: zhujie@cczu.edu.cn)

Abstract: Active macromolecules in *Pleurotus ostreatus* fungi are playing an important role in more and more aspects. Therefore, in this study, low-temperature plasma was used to treat *Pleurotus ostreatus* fungi. The optimal treatment conditions were as follows: treatment power 130 W, treatment time 25 s, and working pressure 140 Pa. Then scanning electron microscopy and polysaccharide composition analysis were carried out.

Keywords: *Pleurotus ostreatus* Fungus; Low-temperature Plasma; Scanning Electron Microscope Analysis; Polysaccharide Component Analysis.

1. Introduction

Pleurotus ostreatus is an edible mushroom that has been widely studied in recent years [1-4] and has certain anti-aging and anti-fatigue functions [5-9]. At present, more and more attention has been paid to some active macromolecules in *Pleurotus ostreatus* fungi [10-12]. The traditional method is to improve the active components in *Pleurotus ostreatus* fungi by optimizing the culture medium [13] and treating the fruiting body [14]. However, the disadvantages of the above method are complicated operation and long time-consuming. At the same time, it may also pollute the environment, and some safety problems appear in biological experiments. Therefore, it is of great significance to find an experimental method that is convenient to use, time-saving and labor-saving, green and pollution-free, and can improve the biological activity of *Pleurotus ostreatus* fungi.

The working principle of low-temperature plasma is that in the plasma chamber, active particles in a high-energy state, such as excited atoms, molecules, reactive oxygen radicals, etc., will produce more complicated physical and chemical reactions when they contact with substances [15-18]. The sample to be treated is put into the cavity, and then the specified gas is introduced, and then the discharge power, pretreatment time, and working pressure are set for treatment.

The existing research results show that low-temperature plasma can promote the synthesis of fungal polysaccharides from *Pleurotus ostreatus* [19,20]. Then analyzing whether the composition of monosaccharide has changed becomes the focus of the next research. Polysaccharide is a natural macromolecule composed of several monosaccharides linked by chemical bonds [21]. The research shows that the main components of monosaccharides are arabinose, xylose, rhamnose, mannose, glucose, galactose, fucose, ferric acid, and so on. There are about 100 different bioactive substances in the fruiting body of *Pleurotus ostreatus*, which is mainly considered a potential new source of dietary fiber [22]. However, fungal cell walls are rich in non-starch polysaccharides, among which β -glucan is the most interesting functional component and phenolic substance [23,24]. Because of the change in structural composition, their activities are significantly different [25-27]. Due to the

complexity of its structure, there are different technical methods to determine its structure, including chemical analysis [28], physical analysis [29], and biological analysis [30]. Researchers can provide a reference for the potential application of polysaccharides in industrial sectors by studying the structure of polysaccharides.

2. Materials and Methods

2.1. Preparation of Reagent

(1) PDA solid culture medium (purchased from Shanghai Bowie) was weighed, and distilled water was added at a ratio of 1:25 [31], which was dissolved in water and sterilized at 121°C for 20 minutes.

(2) According to the requirements of PDB (purchased from Shanghai Bowie), it was added to distilled water at a ratio of 1:40 and sterilized at 121°C for 20 minutes.

2.2. Extraction of Crude Polysaccharide

(1) After being activated, *Pleurotus ostreatus* fungi were inoculated into a PDA culture medium, cultured in a dark incubator at 26°C, and pretreated after 5 days.

(2) Under the optimum conditions of treatment power of 130 W, treatment time of 25 s, and working pressure of 140 Pa, the treated strain was inoculated into a new culture medium.

(3) When the mycelium to be treated is full of Petri dish, it is inoculated into PDB liquid culture medium and then cultured at 26°C 200 r/min for 5 days [32], and the cultured mycelium is collected for later use.

(4) The collected mycelium was dried in an oven at 60°C.

(5) Extracting polysaccharides from dried mycelium to get crude polysaccharides [33,34].

2.3. Purification of Crude Polysaccharide

(1) The extracted crude polysaccharides were weighed and dissolved to prepare a 1% sugar solution.

(2) Papain was added to the sugar solution in a corresponding proportion and stirred in a water bath at 50°C for 1 hour [35].

(3) Cooling to room temperature after the water bath, centrifuging, and removing the precipitate.

(4) The above process is repeated several times until no precipitate is produced after centrifugation.

(5) Use Coomassie Brilliant Blue G-250 to determine the content of protein in the obtained solution. After it meets the requirements, proceed to the next operation [36].

(6) Adding the proper amount of activated carbon powder into the obtained supernatant, stirring to absorb the pigment, and centrifuging to leave the supernatant [37].

(7) Pour the supernatant into a dialysis bag of 200 Da for dialysis, and remove small molecular structures such as salt [38].

(8) Concentrate the obtained polysaccharide solution, then freeze-dried to obtain a relatively pure polysaccharide.

2.4. High-performance Liquid Chromatography Analysis

High-performance liquid chromatography was used to detect polysaccharide components. The ratio of the mobile phase is 0.05 m potassium dihydrogen phosphate solution (adjusted to pH 6.70 with sodium hydroxide solution) and acetonitrile solution, and the flow rate was 1.0 mL/min.

(1) Preparation of control solution: mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, N-acetylglucosamine, glucose, N-acetylgalactosamine, galactose, xylose, arabinose, fucose were used as reference materials, and the reference materials were precisely weighed. It was added to water and diluted to 50 micrograms of the mixed control solution.

(2) Accurately suck 250 mL of mixed solution in a 5 mL EP test tube, add 250 mL of 0.6 mol/L NaOH and 500 mL 0.4 mol/L PMP-methanol, and react for 1 hour. Cool in cold water for 10 minutes. Add 500 uL 0.3 mol/L HCl to neutralize, then add 1 mL of chloroform to vortex for 1 minute, centrifuge at 3000 r/min for 10 minutes, carefully take the supernatant, extract for 3 times, and take the supernatant for high-performance liquid chromatography.

(3) Preparation of determination sample: accurately weigh the sample into a 10 mL ampoule, add 3.0 mL 2 mol/L TFA into the 10 mL ampoule, fill it with nitrogen, seal it, perform acid hydrolysis at 120°C, take it out after 4 hours, add methanol, blow dry TFA with nitrogen, and then dissolve it with 3.0 mL of water.

3. Results and Discussion

3.1. Analysis of Scanning Electron Microscope Results

The mycelia cultured for a certain period was fixed with 2.5% glutaraldehyde specially used for the electron microscope. After fixation, it was dehydrated, then freeze-dried, then fixed on the sample table, and then put into a scanning electron microscope. The surface structure of the mycelium was observed at 5000 times and 15000 times respectively. The results are shown in Figure 1.

Through the comparison of Figure 1, it can be found that under the same magnification, the mycelium of *Pleurotus ostreatus* after low-temperature plasma treatment is more robust, especially at the branch of mycelium, indicating that its growth rate will be more vigorous than that of the control. At the same time, from Table 1, it can be found that the thickness of the treated mycelium under a scanning electron microscope is 1.788 ± 0.11 , while the thickness of the control is 1.587 ± 0.15 , which also proves that the treated mycelium grows more vigorously. After the t-test, it is found that the P

value is $0.00313 < 0.05$, which indicated that there is a significant difference between them.

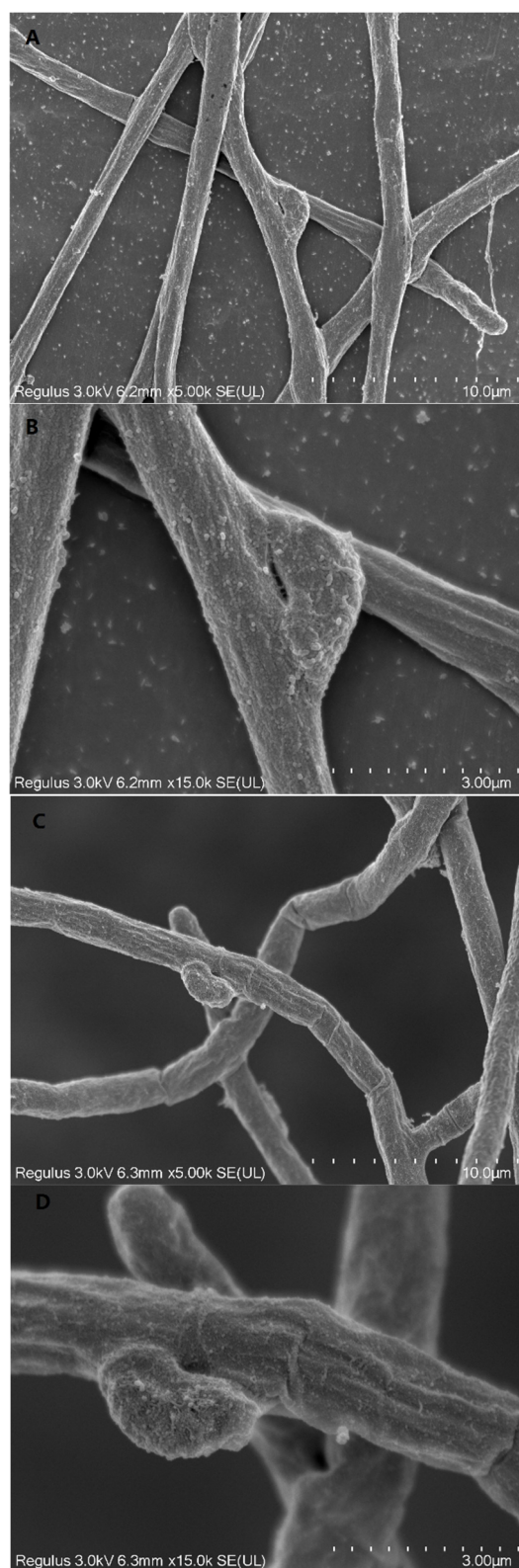


Figure 1. Scanning electron microscope images of *Pleurotus ostreatus* mycelium before and after treatment (A, C: $\times 5000$, B, D: $\times 15000$; A, B: control group, C, D: treatment group)

Table 1. The shape of mycelium before and after treatment

		N	mean value	SD	SEM	mid-value
A		10	1.788	0.10737	0.03395	1.79
B		10	1.587	0.15246	0.04821	1.545
	difference	10	0.201		0.05897	0.235
	in total	20	1.6875	0.16463	0.03681	1.69

(Group A was the treatment group and Group B was the control group)

3.2. Component Analysis of Polysaccharide

Polysaccharide is a kind of natural high molecular substance, and its molecular weight and complex structure make its physiological activities varied. Polysaccharides are widely distributed in fungi and plants, but the structure of polysaccharides will change under different conditions. In addition, the crude polysaccharide also contains protein, pigments, phenols, and some inorganic salt ions, which require a series of operations to become purer. However, different treatment methods have a certain influence on its physiological activity. Generally, the polysaccharide obtained by freeze-drying has better biological activity.

It can be seen from Figure 2 and Table 2 that there are 10 kinds of *Pleurotus ostreatus* polysaccharides in the control group, which are mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, arabinose, and fucose. Together, they constitute the polysaccharide components of *Pleurotus ostreatus* fungi, and their contents are 78.835%, 4.223%, 1.508%, 2.721%, 0.289%, 3.567%, 1.656%, 0.199%, 1.405%, and 5.597% respectively. The content of mannose is the highest, and the other nine monosaccharides are relatively low.

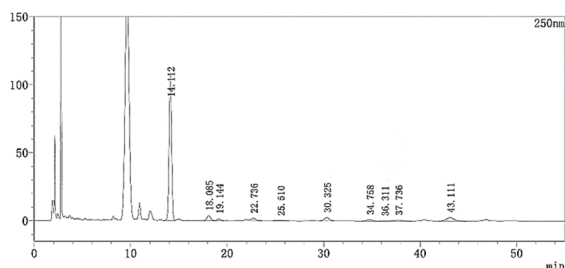


Figure 2. Composition of polysaccharide from untreated *Pleurotus ostreatus*

Table 2. Contents of monosaccharides in polysaccharide of *Pleurotus ostreatus* in the control group

Compound	Retention time	Reserved area	height	area (%)
Mannose	14.112	1941303	107904	78.835
Ribose	18.085	103993	3819	4.223
Rhamnose	19.144	37129	1539	1.508
Glucuronic acid	22.736	66994	1968	2.721
Galactouronic acid	25.510	7128	147	0.289
Glucose	30.325	87847	2441	3.567
Galactose	34.758	40774	1014	1.656
Xylose	36.311	4895	127	0.199
Arabinose	37.736	34598	590	1.405
Fucose	43.111	137831	2466	5.597
		2462491	122015	100.000

In Figure 3 and Table 3, the components of *Pleurotus ostreatus* polysaccharides include monosaccharides such as mannose, ribose, rhamnose, glucuronic acid, glucose, galactose, xylose, arabinose, fucose, and their derivatives. The residence time was 13.749, 17.901, 18.700, 22.301, 29.781, 34.151, 35.884, 37.257, and 42.749 minutes respectively. The content of mannose is the highest, accounting for 58.484%. Glucose and galactose are the second, accounting for 10.997% and 9.729% respectively. The content of glucuronic acid is the lowest, which is only

0.673.

Table 3. Content of monosaccharides in polysaccharides of *Pleurotus ostreatus* in the treatment group

Compound	Retention time	Reserved area	height	area (%)
Mannose	13.749	429537	24120	58.484
Ribose	17.901	20153	886	2.744
Rhamnose	18.700	38352	1700	5.222
Glucuronic acid	22.301	4945	215	0.673
Glucose	29.781	80768	2231	10.997
Galactose	34.151	71457	1765	9.729
Xylose	35.884	49894	1181	6.793
Arabinose	37.257	9264	229	1.261
Fucose	42.749	30081	491	4.096
		734450	32818	100.000

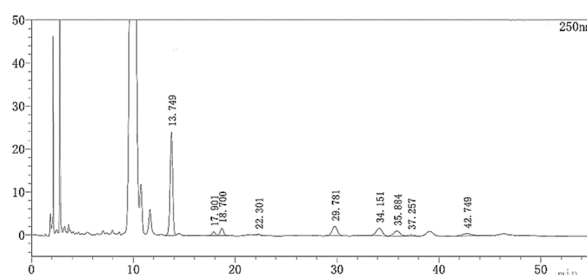


Figure 3. Composition of polysaccharide from *Pleurotus ostreatus* treated by plasma

In contrast, the composition of monosaccharides changed before and after plasma treatment, from 10 in the control group to 9 in the treatment group. Although both of them have the highest mannose content, there is a great difference, and the control group is from 78.835% to 58.484%, which is 20.351% lower. However, the contents of glucose, galactose, and xylose have all increased to a certain extent, and there is not much difference between arabinose and fucose. Especially in the control group, galacturonic acid disappeared after plasma pretreatment. This may be due to the positive biological effect of plasma on *Pleurotus ostreatus* fungi, and the conversion of various monosaccharides was completed through some complicated reactions.

The polysaccharide of *Pleurotus ostreatus* is composed of many monosaccharides. In the process of absorption and digestion, polysaccharides will be hydrolyzed into monosaccharides [39-41], and the functions and functions of monosaccharides are also different. Therefore, the function of polysaccharides is also expressed by monosaccharides. Mannose is also called hexose because it has six carbon atoms and belongs to macromolecular monosaccharide, which is absorbed quickly. It is also the C-2 epimer of glucose, which can be converted into glucose [42]. Mannose mainly exists in the form of manna, hemicellulose, and cellulose in the natural environment. The structure of mannose is similar to that of glucose and fructose [43]. Moreover, mannose has a very important nutritional value, and its sweetness is lower than that of glucose, so it can be used as a sugar substitute. It can also be used as a carbohydrate nutrient to participate in immunomodulation and anti-tumor functions. Mannose has been widely used in industries such as food, medicine, cosmetics, and food additives [44]. Ribose exists in the form of glycoside in cells and furanose in mycelium, which is involved in the synthesis of vitamins and coenzymes. It is also an important part of RNA and participates in the transmission

of genetic material [45]. Rhamnose is 6- deoxy -L- mannose with the molecular formula of $C_6H_{12}O_5$, which can inhibit the growth of harmful bacteria and promote the growth of beneficial bacteria. Moreover, in the process of cell wall synthesis, it is the sugar unit needed to synthesize pectin polymer and cell wall glycoprotein. It can also combine with some metabolites to form ether bonds and carbon-carbon bonds, which are unstable to acids [46-48].

Fucoidan belongs to deoxyhexose, which is used to modify side chains in cell walls, participate in the biosynthesis of various cell wall polymers, and modify sugar-mediated proteins [49,50]. The main function of glucuronic acid is to fix some substances on that cell wall [51]. Glucose is a very common monosaccharide that mainly provides energy for life [52]. Glucose is mostly stored in the form of poly glucan, which is the main component of cellulose synthesis and also the metabolic starting point of carboxylate and amino acid synthesis [53-55]. But the most important thing is that glucose can also be used as a signal molecule to regulate the growth of organisms. Galactose, as a common side chain residue of polysaccharides, plays an important role in many aspects of macromolecules, involving daily metabolism, and it lack will lead to functional disorder. The subtle difference in the fine structure of polysaccharides caused by the loss of galactose residues has far-reaching effects on many macromolecules, such as water solubility and rheological behavior. If galactose does not exist, it will lead to the transformation of the non-sugar unit structure into a pentasaccharide structure [56,57]. Xylose is a kind of xylan, which does not appear in a free state. It is the connecting unit of the sugar chain and serine in some glycoproteins. Xylose is mostly used to produce xylitol, which has the functions of enhancing immunity [58] and resisting fatigue [59]. Arabinose is an organic substance, the chemical formula is $C_5H_{10}O_5$, also known as L (+) - gum aldose, L (+) - pentose, pectin sugar, and so on, which has high application value. It is similar to xylose and rarely occurs in free form. It is mainly combined with other monosaccharides, and heteropolysaccharide exists in colloid, hemicellulose, pectin, and other substances. Therefore, it has good stability. The galacturonic acid peculiar to the control group is the constituent unit of pectin acid, which exists in many plants, such as guava and mung bean [60].

These monosaccharides work together to make *Pleurotus ostreatus* fungi have good medicinal value. Especially several monosaccharides that changed greatly before and after treatment. Mannose can exert immune function and anti-tumor effect in some cases [61]. Galactose can also enhance the metabolism of the human body [62]. These two kinds of monosaccharides with unique functions are epimers of glucose, which can be transformed into each other under certain conditions to better improve the body. Glucose can provide energy for all kinds of life activities. Xylose can be changed into xylitol, which has an obvious anti-fatigue effect. Therefore, plasma treatment influences the composition and monosaccharide content of *Pleurotus ostreatus* fungi, which have high medicinal and nutritional value.

4. Conclusion

Through scanning electron microscope analysis and high-performance liquid chromatography analysis, it can be found that low-temperature plasma treatment of *Pleurotus ostreatus* fungi will have a certain impact on mycelium, and its polysaccharide content will also fluctuate. It is realized by the change of monosaccharide, which changes the content of

monosaccharide. All kinds of monosaccharides play an irreplaceable role in cells, which together constitute the high nutritional value of *Pleurotus ostreatus* polysaccharides.

Author Contributions

Jie Zhu conceived and designed research; Yan Guo, Youjun Wang, and Xiaoyan Xu conducted experiments; Yan Guo wrote the original manuscript; Jie Zhu reviewed and edited the manuscript. All authors read and approved the manuscript.

Data Availability

I, the corresponding author (Jie Zhu), declare on behalf of all the authors that as per the policy of the Journal, the data and material can be made available.

Declarations

Ethical Approval is Not applicable.

Consent to Participate is Not applicable.

Consent to Publish All authors approve the manuscript for publication.

Conflict of Interest The authors declare no competing interests.

Acknowledgments

The Analysis and Testing Center of Changzhou University is also acknowledged.

This research was supported by the National Natural Science Foundation of China (22278040).

References

- [1] Li, C., & Xu, S. (2022). Edible mushroom industry in China: current state and perspectives. *Applied microbiology and biotechnology*, 106(11), 3949–3955. <https://doi.org/10.1007/s00253-022-11985-0>.
- [2] Ma, G., Du, H., Hu, Q., Yang, W., Pei, F., & Xiao, H. (2022). Health benefits of edible mushroom polysaccharides and associated gut microbiota regulation. *Critical reviews in food science and nutrition*, 62(24), 6646–6663. <https://doi.org/10.1080/10408398.2021.1903385>.
- [3] Cerletti, C., Esposito, S., & Iacoviello, L. (2021). Edible Mushrooms and Beta-Glucans: Impact on Human Health. *Nutrients*, 13(7), 2195. <https://doi.org/10.3390/nu13072195>.
- [4] Grimm, D., & Wösten, H. A. B. (2018). Mushroom cultivation in the circular economy. *Applied microbiology and biotechnology*, 102(18), 7795–7803. <https://doi.org/10.1007/s00253-018-9226-8>.
- [5] Li, H., Zhao, H., Gao, Z., Song, X., Wang, W., Yuan, F., Feng, Y., Zhang, Y., Zhang, J., Zhang, S., & Jia, L. (2019). The Antioxidant and Anti-Aging Effects of Acetylated Mycelia Polysaccharides from *Pleurotus Jammer*. *Molecules (Basel, Switzerland)*, 24(15), 2698. <https://doi.org/10.3390/molecules24152698>.
- [6] Bhambri, A., Srivastava, M., Mahale, V. G., Mahale, S., & Karn, S. K. (2022). Mushrooms as Potential Sources of Active Metabolites and Medicines. *Frontiers in microbiology*, 13, 837266. <https://doi.org/10.3389/fmicb.2022.837266>.
- [7] Sun, Y., Hu, X., & Li, W. (2017). Antioxidant, antitumor and immunostimulatory activities of the polypeptide from *Pleurotus eryngii* mycelium. *International journal of biological macromolecules*, 97, 323–330. <https://doi.org/10.1016/j.ijbiomac.2017.01.043>.

- [8] Jiang, X., Chu, Q., Li, L., Qin, L., Hao, J., Kou, L., Lin, F., & Wang, D. (2018). The anti-fatigue activities of *Tuber melanosporum* in a mouse model. *Experimental and therapeutic medicine*, 15(3), 3066–3073. <https://doi.org/10.3892/etm.2018.5793>.
- [9] Sun, H., Shu, F., Guan, Y., Kong, F., Liu, S., Liu, Y., & Li, L. (2023). Study of anti-fatigue activity of polysaccharide from fruiting bodies of *Armillaria gallica*. *International journal of biological macromolecules*, 241, 124611. <https://doi.org/10.1016/j.ijbiomac.2023.124611>.
- [10] Mishra, V., Tomar, S., Yadav, P., & Singh, M. P. (2021). Promising anticancer activity of polysaccharides and other macromolecules derived from oyster mushroom (*Pleurotus sp.*): An updated review. *International journal of biological macromolecules*, 182, 1628–1637. <https://doi.org/10.1016/j.ijbiomac.2021.05.102>.
- [11] Zhang, Y., Zhang, Z., Liu, H., Wang, D., Wang, J., Liu, M., Yang, Y., & Zhong, S. (2022). A natural selenium polysaccharide from *Pleurotus ostreatus*: Structural elucidation, anti-gastric cancer and anti-colon cancer activity in vitro. *International journal of biological macromolecules*, 201, 630–640. <https://doi.org/10.1016/j.ijbiomac.2022.01.101>.
- [12] Khinsar, K. H., Abdul, S., Hussain, A., Ud Din, R., Lei, L., Cao, J., Abbasi, M., Ur Rehman, A., Farooqui, N., Yi, X., Min, H., Wang, L., & Mintao, Z. (2021). Anti-tumor effect of polysaccharide from *Pleurotus ostreatus* on H22 mouse Hepatoma ascites in-vivo and hepatocellular carcinoma in-vitro model. *AMB Express*, 11(1), 160. <https://doi.org/10.1186/s13568-021-01314-5>.
- [13] Liu, Q., Yang, J., Wang, X., Wei, L., & Ji, G. (2021). Effect of culture medium optimization on the secondary metabolites activity of *Lysobacter antibioticus* 13-6. *Preparative biochemistry & biotechnology*, 51(10), 1008–1017. <https://doi.org/10.1080/10826068.2021.1888298>.
- [14] Suzuki, D., Sato, Y., Kamasaka, H., & Kuriki, T. (2020). Effects of Phosphoryl Oligosaccharides of Calcium (POs-Ca) on Mycelial Growth and Fruiting Body Development of the Edible Mushroom, *Pleurotus ostreatus*. *Journal of applied glycoscience*, 67(3), 67–72. https://doi.org/10.5458/jag.jag.JAG-2020_0001.
- [15] Lee, Y., Kim, S., Lee, J., Cho, C., Seong, I., & You, S. (2022). Low-Temperature Plasma Diagnostics to Investigate the Process Window Shift in Plasma Etching of SiO₂. *Sensors (Basel, Switzerland)*, 22(16), 6029. <https://doi.org/10.3390/s22166029>.
- [16] Sebastian, A., Spulber, D., Lisouskaya, A., & Ptasincka, S. (2022). Revealing low-temperature plasma efficacy through a dose-rate assessment by DNA damage detection combined with machine learning models. *Scientific reports*, 12(1), 18353. <https://doi.org/10.1038/s41598-022-21783-3>.
- [17] Cao, Y., Tang, M., Yang, P., Chen, M., Wang, S., Hua, H., Chen, W., & Zhou, X. (2020). Atmospheric Low-Temperature Plasma-Induced Changes in the Structure of the Lignin Macromolecule: An Experimental and Theoretical Investigation. *Journal of agricultural and food chemistry*, 68(2), 451–460. <https://doi.org/10.1021/acs.jafc.9b05604>.
- [18] Wang, X., Liu, Y., Tang, G., Wang, H., & Zhao, Y. (2020). Effects of low-temperature plasma treatment on pulmonary function in children with obstructive sleep apnea-hypopnea syndrome. *Irish journal of medical science*, 189(2), 603–609. <https://doi.org/10.1007/s11845-019-02132-2>.
- [19] Zhang, Y., Wang, Z., Jin, G., Yang, X., & Zhou, H. (2017). Regulating dyslipidemia effect of polysaccharides from *Pleurotus ostreatus* on fat-emulsion-induced hyperlipidemia rats. *International journal of biological macromolecules*, 101, 107–116. <https://doi.org/10.1016/j.ijbiomac.2017.03.084>.
- [20] Chen, L., Zhang, Y., Sha, O., Xu, W., & Wang, S. (2016). Hypolipidaemic and hypoglycaemic activities of polysaccharide from *Pleurotus eryngii* in Kunming mice. *International journal of biological macromolecules*, 93 (Pt A), 1206–1209. <https://doi.org/10.1016/j.ijbiomac.2016.09.094>.
- [21] Melelli, A., Jamme, F., Beaugrand, J., & Bourmaud, A. (2022). Evolution of the ultrastructure and polysaccharide composition of flax fibres over time: When history meets science. *Carbohydrate polymers*, 291, 119584. <https://doi.org/10.1016/j.carbpol.2022.119584>.
- [22] Elkanah, F. A., Oke, M. A., & Adebayo, E. A. (2022). Substrate composition effect on the nutritional quality of *Pleurotus ostreatus* (MK751847) fruiting body. *Heliyon*, 8(11), e11841. <https://doi.org/10.1016/j.heliyon.2022.e11841>.
- [23] Aoe S. (2021). Beta-Glucan in Foods and Health Benefits. *Nutrients*, 14(1), 96. <https://doi.org/10.3390/nu14010096>.
- [24] Wani, S. M., Gani, A., Mir, S. A., Masoodi, F. A., & Khanday, F. A. (2021). β -Glucan: A dual regulator of apoptosis and cell proliferation. *International journal of biological macromolecules*, 182, 1229–1237. <https://doi.org/10.1016/j.ijbiomac.2021.05.065>.
- [25] Tang, Z., & Huang, G. (2022). Extraction, structure, and activity of polysaccharide from *Radix astragali*. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 150, 113015. <https://doi.org/10.1016/j.biopha.2022.113015>.
- [26] Nai, J., Zhang, C., Shao, H., Li, B., Li, H., Gao, L., Dai, M., Zhu, L., & Sheng, H. (2021). Extraction, structure, pharmacological activities and drug carrier applications of *Angelica sinensis* polysaccharide. *International journal of biological macromolecules*, 183, 2337–2353. <https://doi.org/10.1016/j.ijbiomac.2021.05.213>.
- [27] Huang, H., & Huang, G. (2020). Extraction, separation, modification, structural characterization, and antioxidant activity of plant polysaccharides. *Chemical biology & drug design*, 96(5), 1209–1222. <https://doi.org/10.1111/cbdd.13794>.
- [28] Zeng, P., Li, J., Chen, Y., & Zhang, L. (2019). The structures and biological functions of polysaccharides from traditional Chinese herbs. *Progress in molecular biology and translational science*, 163, 423–444. <https://doi.org/10.1016/bs.pmbts.2019.03.003>.
- [29] Iijima, M., Hatakeyama, T., & Hatakeyama, H. (2021). DSC and TMA Studies of Polysaccharide Physical Hydrogels. *Analytical sciences : the international journal of the Japan Society for Analytical Chemistry*, 37(1), 211–219. <https://doi.org/10.2116/analsci.20SAR10>.
- [30] Fan, Y., Zhou, X., & Huang, G. (2022). Preparation, structure, and properties of tea polysaccharide. *Chemical biology & drug design*, 99(1), 75–82. <https://doi.org/10.1111/cbdd.13924>.
- [31] Allieux, F. M., Merhebi, S., Liu, L., Centurion, F., Abbasi, R., Zhang, C., Ireland, J., Biazik, J. M., Mayyas, M., Yang, J., Mousavi, M., Ghasemian, M. B., Tang, J., Xie, W., Rahim, M. A., & Kalantar-Zadeh, K. (2023). A liquid metal-polydopamine composite for cell culture and electro-stimulation. *Journal of materials chemistry. B*, 11(17), 3941–3950. <https://doi.org/10.1039/d2tb02079c>.
- [32] Zhang, W. R., Liu, S. R., Kuang, Y. B., & Zheng, S. Z. (2019). Development of a Novel Spawn (Block Spawn) of an Edible Mushroom, *Pleurotus ostreatus*, in Liquid Culture and its Cultivation Evaluation. *Mycobiology*, 47(1), 97–104. <https://doi.org/10.1080/12298093.2018.1552648>.
- [33] Chen, F., Huang, G., Yang, Z., & Hou, Y. (2019). Antioxidant activity of *Momordica charantia* polysaccharide and its derivatives. *International journal of biological macromolecules*, 138, 673–680. <https://doi.org/10.1016/j.ijbiomac.2019.07.129>.

- [34] Zhang, J., Ye, Z., Liu, G., Liang, L., Wen, C., Liu, X., Li, Y., Ji, T., Liu, D., Ren, J., & Xu, X. (2022). Subcritical Water Enhanced with Deep Eutectic Solvent for Extracting Polysaccharides from *Lentinus edodes* and Their Antioxidant Activities. *Molecules* (Basel, Switzerland), 27(11), 3612. <https://doi.org/10.3390/molecules27113612>.
- [35] Zhong, W., Yang, C., Zhang, Y., & Yang, D. (2022). The prebiotic properties of polysaccharides obtained by differentiated deproteinization methods from *Flos Sophorae Immaturus* on *Lactobacillus fermentum*. *Frontiers in microbiology*, 13, 1007267. <https://doi.org/10.3389/fmicb.2022.1007267>.
- [36] Kielkopf, C. L., Bauer, W., & Urbatsch, I. L. (2020). Bradford Assay for Determining Protein Concentration. *Cold Spring Harbor protocols*, 2020(4), 102269. <https://doi.org/10.1101/pdb.prot102269>.
- [37] Ahmad Farid, M. A., Hassan, M. A., Roslan, A. M., Ariffin, H., Norrahim, M. N. F., Othman, M. R., & Yoshihito, S. (2021). Improving the decolorization of glycerol by adsorption using activated carbon derived from oil palm biomass. *Environmental science and pollution research international*, 28(22), 27976–27987. <https://doi.org/10.1007/s11356-021-12585-7>.
- [38] Azarhava, H., Bajestani, M. I., Jafari, A., Vakilchah, F., & Mousavi, S. M. (2020). Production and physicochemical characterization of bacterial poly gamma- (glutamic acid) to investigate its performance on enhanced oil recovery. *International journal of biological macromolecules*, 147, 1204–1212. <https://doi.org/10.1016/j.ijbiomac.2019.10.090>.
- [39] Zeng, S., Long, J., Sun, J., Wang, G., & Zhou, L. (2022). A review on peach gum polysaccharide: Hydrolysis, structure, properties and applications. *Carbohydrate polymers*, 279, 119015. <https://doi.org/10.1016/j.carbpol.2021.119015>.
- [40] Li, F., Sun, X., Yu, W., Shi, C., Zhang, X., Yu, H., & Ma, F. (2021). Enhanced konjac glucomannan hydrolysis by lytic polysaccharide monoxygenases and generating prebiotic oligosaccharides. *Carbohydrate polymers*, 253, 117241. <https://doi.org/10.1016/j.carbpol.2020.117241>.
- [41] Wolf, M., & Paulino, A. T. (2019). Full-factorial central composite rotational design for the immobilization of lactase in natural polysaccharide-based hydrogels and hydrolysis of lactose. *International journal of biological macromolecules*, 135, 986–997. <https://doi.org/10.1016/j.ijbiomac.2019.06.032>.
- [42] Gonzalez, P. S., O'Prey, J., Cardaci, S., Barthet, V. J. A., Sakamaki, J. I., Beaumatin, F., Roseweir, A., Gay, D. M., Mackay, G., Malviya, G., Kania, E., Ritchie, S., Baudot, A. D., Zunino, B., Mrowinska, A., Nixon, C., Ennis, D., Hoyle, A., Millan, D., McNeish, I. A., ... Ryan, K. M. (2018). Mannose impairs tumour growth and enhances chemotherapy. *Nature*, 563(7733), 719–723. <https://doi.org/10.1038/s41586-018-0729-3>.
- [43] Ščupáková, K., Adelaja, O. T., Balluff, B., Ayyappan, V., Tressler, C. M., Jenkinson, N. M., Claes, B. S., Bowman, A. P., Cimino-Mathews, A. M., White, M. J., Argani, P., Heeren, R. M., & Glunde, K. (2021). Clinical importance of high-mannose, fucosylated, and complex N-glycans in breast cancer metastasis. *JCI insight*, 6(24), e146945. <https://doi.org/10.1172/jci.insight.146945>.
- [44] Gu, R., Liu, H., Hu, M., Zhu, Y., Liu, X., Wang, F., Wu, L., Song, D., & Liu, Y. (2023). D-Mannose prevents bone loss under weightlessness. *Journal of translational medicine*, 21(1), 8. <https://doi.org/10.1186/s12967-022-03870-1>.
- [45] Banfalvi G. (2021). Prebiotic Pathway from Ribose to RNA Formation. *International journal of molecular sciences*, 22(8), 3857. <https://doi.org/10.3390/ijms22083857>.
- [46] Li, S., Chen, F., Li, Y., Wang, L., Li, H., Gu, G., & Li, E. (2022). Rhamnose-Containing Compounds: Biosynthesis and Applications. *Molecules* (Basel, Switzerland), 27(16), 5315. <https://doi.org/10.3390/molecules27165315>.
- [47] De Coen, R., Nuhn, L., Perera, C., Arista-Romero, M., Risseuw, M. D. P., Freyn, A., Nachbagauer, R., Albertazzi, L., Van Calenbergh, S., Spiegel, D. A., Peterson, B. R., & De Geest, B. G. (2020). Synthetic Rhamnose Glycopolymer Cell-Surface Receptor for Endogenous Antibody Recruitment. *Biomacromolecules*, 21(2), 793–802. <https://doi.org/10.1021/acs.biomac.9b01483>.
- [48] Daubenspeck, J. M., Liu, R., & Dybvig, K. (2016). Rhamnose Links Moonlighting Proteins to Membrane Phospholipid in *Mycoplasmas*. *PLoS one*, 11(9), e0162505. <https://doi.org/10.1371/journal.pone.0162505>.
- [49] Becker, D. J., & Lowe, J. B. (2003). Fucose: biosynthesis and biological function in mammals. *Glycobiology*, 13(7), 41R–53R. <https://doi.org/10.1093/glycob/cwg054>.
- [50] Schneider, M., Al-Shareffi, E., & Haltiwanger, R. S. (2017). Biological functions of fucose in mammals. *Glycobiology*, 27(7), 601–618. <https://doi.org/10.1093/glycob/cwx034>.
- [51] García-Oliva, C., Cabanillas, A. H., Perona, A., Hoyos, P., Rumbero, Á., & Hernáiz, M. J. (2020). Efficient Synthesis of Muramic and Glucuronic Acid Glycodendrimers as Dengue Virus Antagonists. *Chemistry (Weinheim an der Bergstrasse, Germany)*, 26(7), 1588–1596. <https://doi.org/10.1002/chem.201903788>.
- [52] Bose, S., & Le, A. (2018). Glucose Metabolism in Cancer. *Advances in experimental medicine and biology*, 1063, 3–12. https://doi.org/10.1007/978-3-319-77736-8_1.
- [53] Dienel G. A. (2019). Brain Glucose Metabolism: Integration of Energetics with Function. *Physiological reviews*, 99(1), 949–1045. <https://doi.org/10.1152/physrev.00062.2017>.
- [54] DiNuzzo M. (2019). How glycogen sustains brain function: A plausible allosteric signaling pathway mediated by glucose phosphates. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 39(8), 1452–1459. <https://doi.org/10.1177/0271678X19856713>.
- [55] Von Ah Morano, A. E., Dorneles, G. P., Peres, A., & Lira, F. S. (2020). The role of glucose homeostasis on immune function in response to exercise: The impact of low or higher energetic conditions. *Journal of cellular physiology*, 235(4), 3169–3188. <https://doi.org/10.1002/jcp.29228>.
- [56] Wang, H. H., Sun, Y. N., Qu, T. Q., Sang, X. Q., Zhou, L. M., Li, Y. X., & Ren, F. Z. (2022). Nobiletin Prevents D-Galactose-Induced C2C12 Cell Aging by Improving Mitochondrial Function. *International journal of molecular sciences*, 23(19), 11963. <https://doi.org/10.3390/ijms231911963>.
- [57] Yu, K. M., Yang, P., Huang, T. Y., Shen, T. Y., Lau, J. Y., & Hu, O. Y. (2022). A novel galactose electrochemical biosensor intended for point-of-care measurement of quantitative liver function using galactose single-point test. *Analytical and bioanalytical chemistry*, 414(14), 4067–4077. <https://doi.org/10.1007/s00216-022-04051-1>.
- [58] Liu, X., Lin, Q., Yan, Y., Peng, F., Sun, R., & Ren, J. (2019). Hemicellulose from Plant Biomass in Medical and Pharmaceutical Application: A Critical Review. *Current medicinal chemistry*, 26(14), 2430–2455. <https://doi.org/10.2174/0929867324666170705113657>.
- [59] Rabago, D., Kille, T., Mundt, M., & Obasi, C. (2020). Results of a RCT assessing saline and xylitol nasal irrigation for CRS and fatigue in Gulf War illness. *Laryngoscope investigative otolaryngology*, 5(4), 613–620. <https://doi.org/10.1002/lio2.425>.
- [60] Mariette, A., Kang, H. S., Heazlewood, J. L., Persson, S., Ebert, B., & Lampugnani, E. R. (2021). Not Just a Simple Sugar:

- Arabinose Metabolism and Function in Plants. *Plant & cell physiology*, 62(12), 1791–1812. <https://doi.org/10.1093/pcp/pcab087>.
- [61] Jiang, N., Wang, Y., Zhou, J., Zheng, R., Yuan, X., Wu, M., Bao, J., & Wu, C. (2020). A novel mannose-binding lectin from *Liparis nervosa* with anti-fungal and anti-tumor activities. *Acta biochimica et biophysica Sinica*, 52(10), 1081–1092. <https://doi.org/10.1093/abbs/gmaa090>.
- [62] Conte, F., van Buuringen, N., Voermans, N. C., & Lefeber, D. J. (2021). Galactose in human metabolism, glycosylation and congenital metabolic diseases: Time for a closer look. *Biochimica et biophysica acta. General subjects*, 1865(8), 129898. <https://doi.org/10.1016/j.bbagen.2021.129898>.